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BLOCKADE OF SYMPATHETIC β-RECEPTORS IN THE MYOCARDIAL CIRCULATION

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Considerable confusion still exists concerning the action of cardiac sympathetic nerve stimulation and of its supposed mediators, adrenaline and noradrenaline, on the vasculature of the myocardium. The general consensus of opinion is that they produce coronary vasodilatation. The disagreement is over the mechanism by which this vasodilatation is produced since not only do cardiac sympathetic nerve stimulation and injections of adrenaline and noradrenaline directly affect vascular tone, but they also modify other factors which influence the state of the myocardial vascular bed (aortic perfusion pressure, heart rate, cardiac metabolism and extravascular support). The problem is whether the direct effect of adrenaline and noradrenaline on the myocardial vessels is vasodilator or vasoconstrictor, the latter effect being masked in the intact animal by mechanical and metabolic factors which lead to vasodilatation. Associated with this problem is the question of whether the sympathetic nerves to the vessels are vasoconstrictor or vasodilator and of whether sympathetic tone exists in this vascular bed.

Very little seems to have been done to investigate these problems using substances which block receptor sites. Hashimoto, Shigei, Imai, Saito, Yago, Uei & Clark (1960) found that coronary flow in isolated hearts taken from dogs treated with phenoxybenzamine was greater than in hearts taken from untreated dogs, a finding which can be interpreted as indicating a degree of vasoconstrictor tone for the coronary vessels. It is known, however, that phenoxybenzamine causes the liberation of catechol amines from the adrenal medulla (Millar, Keener & Benfey, 1959) and the high plasma levels of these substances present when the animals were killed would provide an alternative explanation for the increased values for coronary flow obtained. In intact animals, Grayson & Mendel (1961) found that phentolamine, which blocks sympathetic α-receptors, increased myocardial blood flow and decreased myocardial vascular resistance. These effects could similarly be explained by a reduction of sympathetic vasoconstrictor tone although they could also result from the direct vasodilator action which this compound is known to possess.

More recently, compounds which block β-receptors have been produced and one of them, pronethalol (Black & Stephenson, 1962), has been given a trial in the treatment of angina pectoris (Alleyne, Dickinson, Dornhorst, Fulton, Green, Hill, Hurst, Laurence, Pilkington, Prichard, Robinson & Rosenheim, 1963). The present experiments examined the effect of pronethalol on the myocardial circulation and investigated the actions of catechol amines which affect α- and/or β-receptors (noradrenaline, adrenaline, and isoprenaline) before and after blocking the β-receptors.

TABLE 1

EFFECT OF 10-MIN INTRAVENOUS INFUSIONS OF ISOPRENALINE ON BLOOD PRESSURE, HEART RATE, MYOCARDIAL BLOOD FLOW AND CALCULATED MYOCARDIAL VASCULAR RESISTANCE, BEFORE AND AFTER PRONETHALOL (5 MG/KG)

Values are the means and standard errors of the maximum responses during the infusion period (expressed as percentages of the preinfusion levels). * Significant at $P<0{\cdot}001$; † at $P<0{\cdot}01$

Isoprenaline dose (μg/kg/min)	No. of expts.	Percentage change from controls: Before pronethalol — Blood pressure	Heart rate	Blood flow	Vascular resist.	After pronethalol — Blood pressure	Heart rate	Blood flow	Vascular resist.
0·1	15	−18·6 (±2·5)	+15·6 (±1·5)	+36 (±6·4)	−37 (±2·6)	−2·4* (±0·7)	+3·8* (±0·9)	+14·4† (±6·8)	−12* (±4·5)
0·25	13	−29·2 (±2·6)	+22 (±1·7)	+69·5 (±9·3)	−53·7 (±3·7)	−6·2* (±1·5)	+9·5* (±0·7)	+32† (±11·6)	−25* (±4·1)

METHODS

Nineteen mongrel dogs, of either sex and weighing between 6.5 and 11 kg, were used and were anaesthetized with pentobarbitone sodium (40 mg/kg, intraperitoneally). Blood pressure was measured from a polyethylene cannula in a femoral artery using a mercury manometer or a Shillingford-Muller transducer and recorded on one oscillator channel of a Cambridge recording camera. Myocardial blood flow was measured using the method of Grayson & Mendel (1961), which depends upon the thermoelectric measurement of thermal conductivity. The photographic records of myocardial temperature were also analysed to obtain indications of local metabolic heat production (" corrected temperature ") as described by Dosekun, Grayson & Mendel (1960).

Myocardial vascular resistance was calculated whenever blood flow measurements were made from the formula, R=blood pressure (mm Hg) divided by blood flow (Δk, the thermal conductivity of the living heart minus the thermal conductivity of the dead heart). Details of the operative procedure and analysis of results have been described fully in a previous paper (Parratt, 1964).

The drugs used were (±)-isoprenaline hydrochloride (Mann Research Laboratories), (−)-noradrenaline bitartrate (Winthrop), (−)-adrenaline bitartrate and pronethalol hydrochloride (Alderlin, I.C.I.). The doses of adrenaline and noradrenaline refer to the base; those of isoprenaline and pronethalol to the salts. Drugs were given by infusion, using a continuous-injection apparatus delivering 0.5 ml./min, through a polyethylene cannula inserted in a femoral vein. Initial values of blood pressure and flow were obtained during infusions of 0.9% saline.

RESULTS

The effects of pronethalol on the myocardial circulation

Pronethalol was given intravenously in a concentration of 5 mg/ml. so that a dosage of 5 mg/kg body weight had been infused in 25 to 30 min. Immediately after the completion of the infusion the arterial blood pressure was reduced by 11%, the heart rate by 18% and the myocardial blood flow by 29% (mean values from fifteen experiments). In all except two experiments β-receptor blockade produced by pronethalol resulted in an increase in resistance to flow in the myocardial vascular bed, the mean value from all the experiments being 32%. Myocardial temperature rose during the infusion period more than one would expect from the decrease in blood flow alone and this increase in " corrected temperature," which averaged 0.1° C, is interpreted as being due to an alteration in myocardial metabolism (see Dosekun, Grayson & Mendel, 1960; Parratt, 1964).

The effects of pronethalol on the response of the myocardial circulation to infusions of isoprenaline, adrenaline and noradrenaline

Isoprenaline. This was particularly effective in increasing myocardial blood flow (Fig. 1) and in decreasing myocardial vascular resistance (Table 1). These changes were accompanied by increase of heart rate and fall of blood pressure which seldom recovered to any extent during the infusion period (Fig. 3). Slight decreases, ranging from −0.03 to 0.19° C, were also obtained in " corrected temperature."

These changes evoked by isoprenaline infusions on blood pressure, heart rate, blood flow and vascular resistance were much reduced by previous treatment with pronethalol. It is clear from Table 1 that the dose of pronethalol used (5 mg/kg) was not sufficient completely to block all β-receptors; nevertheless it significantly altered each of the vascular responses produced by isoprenaline. On several occasions, however, some increase in myocardial blood flow was obtained despite almost complete abolition of the blood pressure

TABLE 2

EFFECT OF 10-MIN INTRAVENOUS INFUSIONS OF ADRENALINE ON BLOOD PRESSURE, HEART RATE, MYOCARDIAL BLOOD FLOW AND CALCULATED MYOCARDIAL VASCULAR RESISTANCE, BEFORE AND AFTER PRONETHALOL (5 MG/KG)

Values indicate maximum responses during the infusion, expressed as percentages of preinfusion levels. *After bilateral vagotomy, where two values are given the response was diphasic

Adrenaline dose (μg/kg/min)	Expt. No.	Percentage change from controls: Before pronethalol — Blood pressure	Heart rate	Blood flow	Vascular resist.	After pronethalol — Blood pressure	Heart rate	Blood flow	Vascular resist.
0·1	3	+4	0	—	—	+63	0	—	—
0·1	4	−4	+5	+69	−43	+61	−40	+64	−24, +15
0·1	5	+9	−3	−5	+5	+68	−11	+58	+29
0·1	11	0	0	−13	+14	—	—	—	—
0·1	8	+10	+3	−24, +3	+10, −16	+39	−26	+67	+30, −2
0·1	6	0	0	+11	−11	+53	−72	+62	+7, −8
0·1	6					+70*	0	+56	+13
0·1	7	+5	+3	−37	+65	+92	−29	−44, +5	+174
0·1	12	−4, +5	+1	+12, −35	−28, +62	+59	−24	−23	+109
0·1	12					+49*	−3	−68	+459
0·1	13	+8	−3	+12	−14	+38	−30	+11, −14	+52
0·1	14	+10	0	+23	−12, +3	+60	+1	−2	+88
0·1	15	+4	+5	+25	−17	+47	+5	+27	+48
	Mean	+4	+1			+58 (+59*)	−23 (−1*)		
0·25	1	−3	−4	+23	−18	+55	−36	+155	−23
0·25	1					+80*	−15	+38	+29
0·25	2	+4	−10	+24	−18	+57	−30	+37	−3, +49
0·25	2					+58*	+7	+79	+40, −16
0·25	3	+20	−5	—	—				
0·25	4	+28	+4	+44	+6, −11				
0·25	5	+37	−4	+23	+30				
0·25	6	+11	−3	+12	−9				
0·25	8	+28	−11	+41	+41, −10	+28	−49	+73	+26, −29
0·25	12	+20	−7	+30	−27, +20				
0·25	13	+24	−6	+20	+27				
0·25	14	+29	−6	+28	−10, +29				
0·25	15	+13	−3	−32, +9	+55, −18				
	Mean	+19	−5·5			+47 (+69*)	−38 (+4*)		

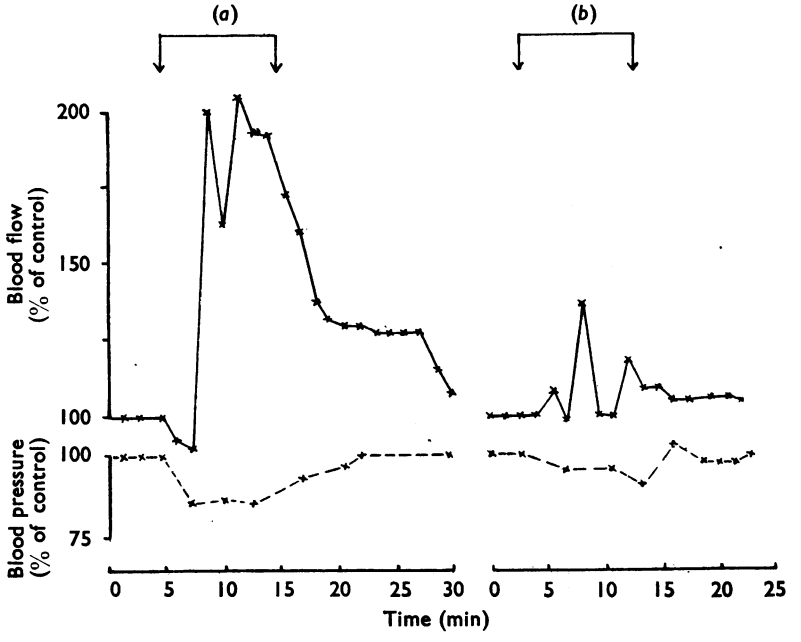

Fig. 1. The effect of 10-min intravenous infusions of isoprenaline (0.25 μg/kg/min) on myocardial blood flow (×——×) and arterial blood pressure (×- - - -×) in a dog. Between (*a*) and (*b*) a slow intravenous infusion of pronethalol (5 mg/kg) was given. Myocardial blood flow and arterial blood pressure are expressed as percentages of the control levels immediately before each isoprenaline infusion.

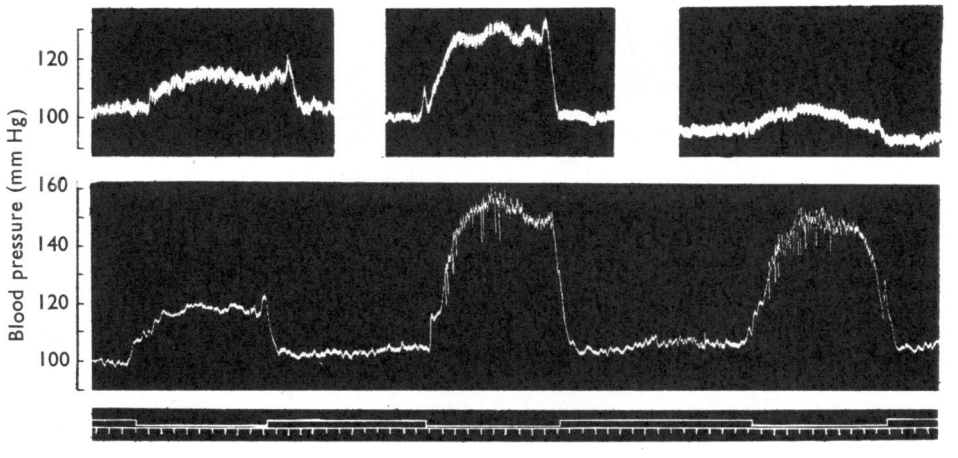

Fig. 2. Dog, 9 kg. Comparison of the effects on arterial blood pressure of intravenous infusions of (from left to right) noradrenaline (0.125 μg/kg/min and 0.25 μg/kg/min) and adrenaline (0.1 μg/kg/min) before (upper records) and after (lower records) a slow infusion of pronethalol (5 mg/kg). Time in minutes.

TABLE 3

EFFECT OF 10-MIN INTRAVENOUS INFUSIONS OF NORADRENALINE ON BLOOD PRESSURE, HEART RATE, MYOCARDIAL BLOOD FLOW AND CALCULATED MYOCARDIAL VASCULAR RESISTANCE, BEFORE AND AFTER PRONETHALOL (5 MG/KG)

Values indicate maximum responses during the infusion, expressed as percentages of the preinfusion levels. Where two values are given the response was diphasic

Noradrenaline dose (μg/kg/min)	Expt. No.	Percentage change from controls: Before pronethalol — Blood pressure	Heart rate	Blood flow	Vascular resist.	After pronethalol — Blood pressure	Heart rate	Blood flow	Vascular resist.
0·125	3	+22	−6						
0·125	4	+13	−4	+18, −14	−6, +3	+29	0	−38	+69
0·125	6	+20	−5	−10	+30	+15	0	−10	+26
0·125	7	+9	−7	+9	+12	+11	0	−7, +3	+21
0·125	12	+8	−4	+73	−38	+23	−2	+31	−13, +23
0·125	13	+9	−6	+3	+11	+16	0	−13	+30
0·125	14	+13	−5	+6	+7	+24	−1	+12	+20
0·125	14	+19	−7	+31	+29, −9				
0·125	15	+13	−4	+24	+16. −9				
0·125	15	+6	+2	+13	−6, +2	+12	0	+12	+21
	Mean	+13	−5	+17		+18	0	0	
0·250	12	+16	−5	+50	−28, +39	+56	−29	−46	+190
0·250	13	+31	−20	+25	−13, +20	+46	−31	+37	+37
0·250	14					+59	−21	+12	+58
	Mean	+23	−12	+37		+54	−27	0	

and heart rate changes. It will also be noted that the modification of the blood flow response is not as significant as the modification of either heart rate or blood pressure.

Adrenaline. The effects of adrenaline on the myocardial circulation were variable (Table 2). With doses which had little, if any, effect on blood pressure or heart rate, blood flow usually increased, indicating a reduction in myocardial vascular resistance. With larger doses (0.25 μg/kg/min), although blood flow was consistently raised, vascular resistance was invariably increased, at least at some stage of the infusion period. It is possible therefore that these blood flow changes are related to the increased perfusion pressure. It will be noted that the dose of adrenaline used did not significantly alter heart rate.

β-Blockade (which, as noted in the isoprenaline experiments, was incomplete) strikingly altered the cardiovascular effects of adrenaline. The pressor effect was greatly enhanced (Fig. 2) and was accompanied by large decreases of heart rate. The bradycardia was abolished by bilateral vagotomy. In general, the increases in blood flow through the myocardium evoked by adrenaline infusions were enhanced by β-blockade and the vascular resistance changes were strikingly altered. Whereas with small doses of adrenaline vascular resistance was usually reduced, after β-blockade adrenaline markedly increased myocardial vascular resistance.

Noradrenaline. The results with noradrenaline are given in Table 3. Before pronethalol infusions of noradrenaline always increased myocardial blood flow and usually also increased myocardial vascular resistance, which indicates that noradrenaline constricts the myocardial vascular bed. After pronethalol the increase in vascular resistance due to

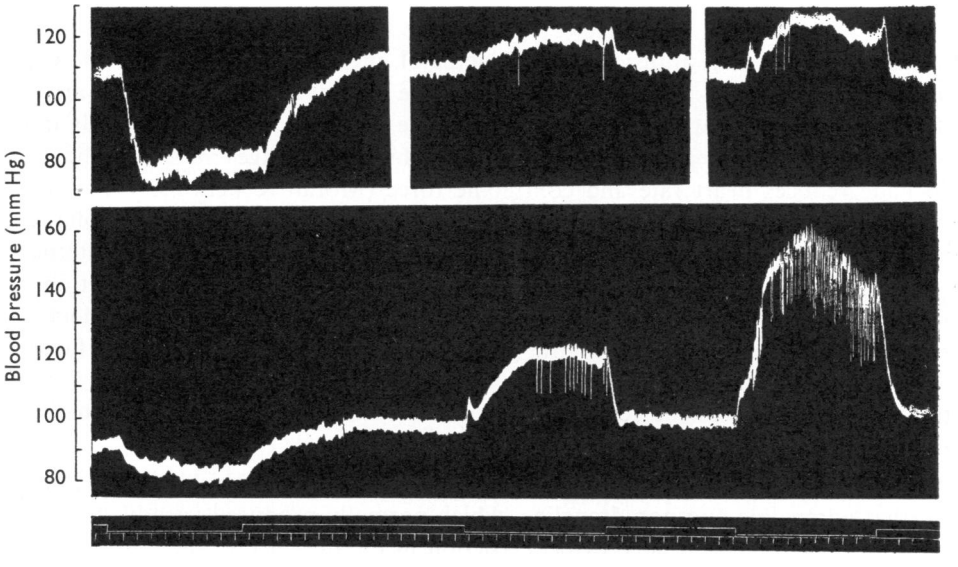

Fig. 3. Dog, 6.5 kg. Effect on arterial blood pressure of intravenous infusions of (from left to right) isoprenaline (0.25 μg/kg/min) and noradrenaline (0.125 μg/kg/min and 0.25 μg/kg/min) before (upper records) and after (lower records) a slow infusion of pronethalol (5 mg/kg). Time in minutes.

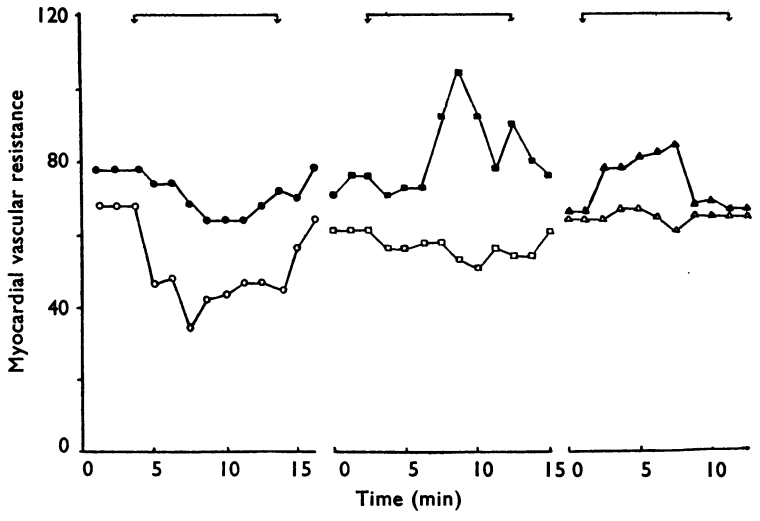

Fig. 4. The effects of 10-min intravenous infusions of (from left to right) isoprenaline (0.1 μg/kg/min), adrenaline (0.1 μg/kg/min) and noradrenaline (0.125 μg/kg/min) on calculated myocardial vascular resistance (in arbitrary units) before (empty symbols) and after (filled symbols) β-blockade with pronethalol (5 mg/kg).

infusion of noradrenaline was significantly enhanced (Fig. 4). The blood pressure response to noradrenaline was also sometimes potentiated after β-blockade, especially with the larger doses of noradrenaline (compare Figs. 2 and 3).

DISCUSSION

In these experiments, infusions of pronethalol increased resistance to flow in the myocardial vascular bed. There are several possible explanations for this. One arises from the fact that extravascular factors such as heart rate and contractile force greatly influence myocardial blood flow, partly, if not entirely, through changes in metabolic activity. β-Blockade reduces heart rate and contractile force (Moran & Perkins, 1958; Black & Stephenson, 1962) and these changes could lead to an alteration in the metabolic requirements of the myocardium with subsequent alterations in vascular tone. Alternatively, the increase in vascular resistance after pronethalol could be due to vasoconstriction arising either from a direct action of the drug on vascular smooth muscle or from β-blockade. It is not possible to determine from the present experiments which of these factors are responsible for the increase in vascular resistance. If it resulted mainly from β-blockade in the vessels themselves the results would indicate sympathetic vasodilator tone to the myocardium.

In their experiments on β-blockade in dog isolated hearts, Hashimoto *et al.* (1960) found that adrenaline and noradrenaline, which normally produced vasodilatation in this preparation, produced coronary vasoconstriction in the presence of dichloroisoprenaline; they concluded that " the sympathomimetic amines cause coronary vasodilatation through a myocardial metabolic mechanism." The fact that coronary vasodilatation induced by adrenaline and noradrenaline is reversed to coronary vasoconstriction by dichloroiso-

prenaline was attributed to " blockade of the metabolic effects of these compounds." Another possibility, however, is that, besides the sympathetic β-receptors known to occur in cardiac contractile tissue, there may be similar receptors in the walls of the myocardial vessels. Treatment with dichloroisoprenaline (or pronethalol) might block these receptors and thus enhance the effects of substances which also influence the α-receptors in the myocardial vasculature.

It seems a reasonable conclusion from the experiments reported here that β-receptors do in fact occur in the walls of the myocardial vessels. Isoprenaline in very small doses increases myocardial flow and decreases myocardial vascular resistance and these effects can be prevented by pronethalol. The strong effect of isoprenaline on coronary blood flow has been reported before. Thus Denison, Bardhanabaedya & Green (1956) and Lewis, Coffman & Gregg (1961) have shown that intracoronary injections of isoprenaline in dogs increase flow and this was attributed, at least in part, to decreased vascular resistance. Denison *et al.* (1959) pointed out that the dilatation occurred before any change in heart contractility or rate. Intravenous infusions of isoprenaline increase myocardial blood flow also in rabbits (Mendel, personal communication) and this, too, was associated with a decrease in vascular resistance. In other vascular beds decreases in resistance due to isoprenaline have been attributed to a direct action on the smooth muscle of the blood vessel wall (Bowman, 1959; Dornhorst & Robinson, 1962). A similar explanation seems reasonable for the action of isoprenaline on the myocardial circulation. It is true that part of the vasodilator action elicited by this amine may be secondary to its effects on cardiac metabolism but it does not seem valid to conclude, as Hashimoto *et al.* (1960) do, that " the presence of pure sympathetic vasodilator receptors (in the heart) is improbable."

The experiments with adrenaline and noradrenaline after β-blockade clearly also demonstrate the existence of α-receptors in the myocardial vascular bed. This is perhaps best illustrated in Fig. 4. Before blockade, infusions of isoprenaline and adrenaline decreased myocardial vascular resistance and this is attributed mainly to an action on vascular β-receptors. The dose of adrenaline used had no chronotropic or inotropic effects and thus it is unlikely that the vasodilatation resulted from a metabolic action of this amine, especially as there was no change in " corrected temperature." Noradrenaline, on the other hand, was slightly vasoconstrictor. After β-blockade, the resistance changes induced by isoprenaline were reduced, those to adrenaline were reversed and those to noradrenaline were potentiated. This is interpreted as meaning that adrenaline and noradrenaline were then causing vasoconstriction by an action on the α-receptors in the walls of the myocardial vessels.

One other very striking change in the cardiovascular effects of adrenaline after β-blockade was the considerable potentiation of the pressor response. Doses of adrenaline which had had hardly any effect on the arterial blood pressure before pronethalol increased the mean pressure by up to 87 mm Hg after pronethalol. This is presumably due to stimulation of α-receptors in the blood vessel walls of the skin and gastrointestinal tract, but it is surprising to find that the effect is so great. From Fig. 2 it can be seen that after pronethalol, adrenaline (0.1 μg/kg/min) was only slightly less effective in raising the blood pressure than noradrenaline (0.25 μg/kg/min), whereas before this dose of adrenaline had been considerably less active than had 0.125 μg/kg/min of noradrenaline. Thus adrenaline was two- to three-times more potent than noradrenaline in stimulating α-receptors. It is interesting to note

that this potentiation of the adrenaline pressor response after pronethalol is not seen in cats (Ariëns, Waelen, Sonneville & Simonis, 1963). Thus there are species variations in the relative activity of adrenaline on α- and β-receptors in the blood vessel walls.

On some occasions the pressor response to noradrenaline, too, was potentiated after β-blockade (Fig. 3), although this was not nearly so clear as for adrenaline (Fig. 2). These results, and other evidence that noradrenaline can sometimes produce vasodilatation (Burn & Hutcheon, 1949; McDowall, 1950; West, 1951), can be explained if noradrenaline has some effect on β-receptors in vascular tissue.

SUMMARY

1. The effects on the dog myocardial circulation of infusions of adrenaline, noradrenaline and isoprenaline have been studied before and after β-blockade with pronethalol, using a heated thermocouple technique.

2. Before β-blockade all three amines increased myocardial blood flow. Isoprenaline always decreased myocardial vascular resistance and adrenaline usually did; noradrenaline increased it.

3. Pronethalol itself decreased myocardial blood flow and increased myocardial vascular resistance. It also modified the actions of each of the three amines. The effect of isoprenaline on myocardial vascular resistance was reduced, that of adrenaline reversed and that of noradrenaline potentiated.

4. β-Blockade potentiated the pressor effects of adrenaline and, to a lesser extent, noradrenaline on the systemic blood pressure.

5. These results are interpreted as indicating the presence of sympathetic β-receptor sites in the myocardial vasculature.

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